

CLAIMS

1. Method to evaluate the integrity of chromatin/DNA and animal sperm comprising:
 - 5 a) a treatment step of the sample containing the sperm, with a solution of DNA denaturing solution,
 - b) a single treatment step with a lysis solution to extract the nuclear proteins,
 - 10 c) an evaluation stage of the integrity of the chromatin/DNA of the sperm characterised because the lysis solution does not contain protein denaturing detergents and essentially does not destroy the tails of the sperm.
- 15 2. Method according to claim 1, characterised in that stage a) precedes that of b), or it only proceeds to b) and c).
3. Method according to claim 1 or 2, characterised in that the lysis solution comprises of a non-ionic non protein denaturing detergent.
- 20 4. Method according to claims 1-3, characterised in that the non ionic detergent is selected from the group toctylphenoxypolyethoxyethanol (Triton X-100), N , N-bis(3-D-Gluconamidopropyl) cholamide (bigCHAP), Brij(r) 35 P, N-decanoyl-N-methylglucamine, digitonin, dodecanoyl-N-methylglucamide, heptanoyl-N- methylglucamide, branched octylphenoxy poly (ethyleneoxy)
 - 25 ethanol (Igepal CA-630), N-Nonanoyl-N-methylglucamine, Nonidet P 40, N-Octanoyl-N-methylglucamine, Span 20 solution, Polysorbate 20 (Tween 20) and their mixtures, preferably Triton X-100.
- 30 5. Method according to claims 1-4, characterised in that the lysis solution comprises sodium chloride between 1 and 3M, dithiothreitol (DTT) between 0.001 and 2M, 2-amino-2 (hydroxymethyl)-1,3-propanediol (Tris) between 0.001M and 2 M and Triton X-100 between 0.1% and 3%.
- 35 6. Method according to claims 1-5, characterised in that the lysis solution comprises 2.5M sodium chloride, around 0.2M DTT, around 0.2M Tris, around 1% Triton X-100 and a pH of around 7.5.
7. Method according to claims 1-6, characterised in that the DNA denaturing solution is acid.

8. Method according to claim 7, characterised in that the DNA denaturing solution comprises an acid selected from the hydrochloric, acetic, nitric acid group or mixtures of these.
- 5 9. Method according to claim 8, characterised in that the DNA denaturing solution comprises hydrochloric acid.
- 10 10. Method according to claims 1-9, characterised in that after steps a) and b) there is a sample staining step.
- 11 11. Method according to claim 10, characterised in that the staining is made with a Wright type solution.
- 15 12. Method according to claims 1-11, characterised in that the sample containing the sperm is included in a medium similar to a suspension, preferably in a microgel.
- 20 13. Method according to claim 12, characterised in that the sample containing the sperm is included in an agarose microgel.
- 25 14. Kit for the evaluation of the quality of the sperm of animals which comprises:
a) a DNA denaturing solution,
b) a lysis solution to extract nuclear proteins,
- 30 characterised in that the lysis solution does not contain a protein denaturing detergent and essentially does not destroy the tails of the sperm.
15. Kit according to claim 14, characterised in that the lysis solution comprises sodium chloride between 1M and 3M , dithiothreitol (DTT) between 0.001M and 2 M, 2-amino-2 (hydroxymethyl)-1,3 propanediol (Tris) between 0.001M and 2 M and Triton X-100 between 0.1% and 3%.